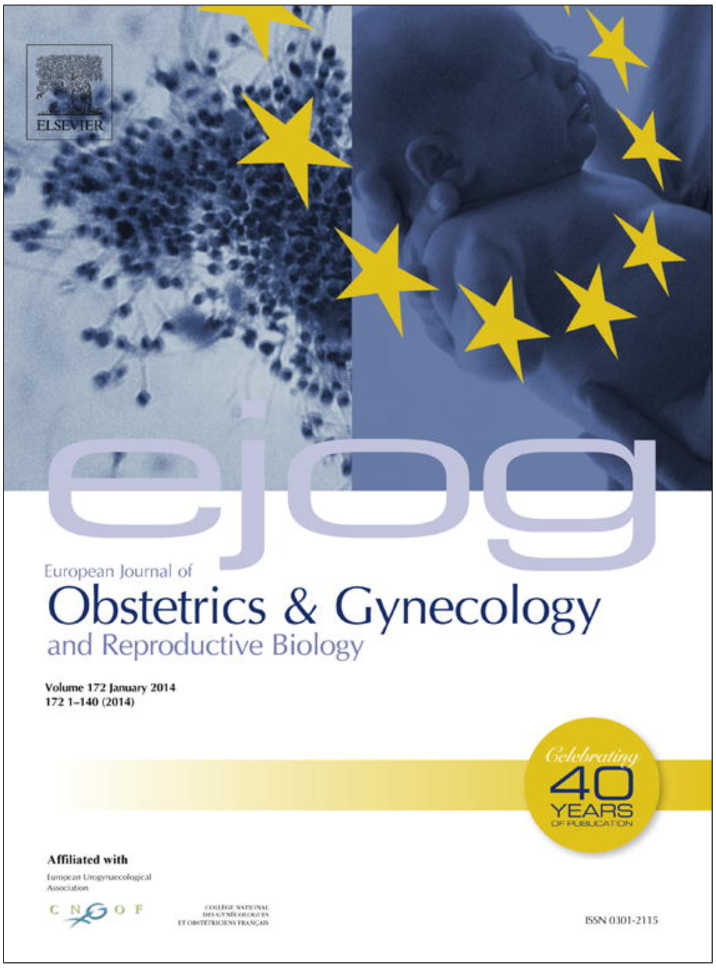
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Effect of treatment of vitamin D deficiency and insufficiency during pregnancy on fetal growth indices and maternal weight gain: a randomized clinical trial

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ABSTRACT

Objective: To determine whether treatment of low serum vitamin D in pregnant women improves fetal growth indices.
Study design: In this open-label randomized clinical trial, 130 Iranian pregnant women (24–26 weeks of gestation) with vitamin D deficiency or insufficiency [25(OH)D <30 ng/ml] were divided at random into an intervention group and a control group. The control group received 200 mg calcium plus a multivitamin (containing vitamin D3 400 U) each day, and the intervention group received 200 mg calcium plus a multivitamin (containing vitamin D3 400 U) each day, plus vitamin D_3 (50,000 U) each week for 8 weeks. At delivery, maternal and cord blood 25(OH)D levels, maternal weight gain, neonatal length, neonatal weight and neonatal head circumference were compared between two groups. Serum vitamin D was measured using enzyme-linked immunosorbent assay. A multivariate regression analysis was performed to examine the independent effect of maternal vitamin D level on fetal growth indices.
Results: Mean (±standard deviation) length (intervention group: 49 ± 1.6 cm; control group: 48.2 ± 1.7 cm; $p = 0.001$), head circumference (intervention group: 35.9 ± 0.7 cm; control group: 35.3 ± 1.0 cm; $p = 0.001$) and weight (intervention group: 3429 ± 351.9 g; control group: 3258.8 ± 328.2 g; $p = 0.01$) were higher in the intervention group compared with the control group. Mean maternal weight gain was higher in the intervention group compared with the control group (13.3 ± 2.4 kg vs 11.7 ± 2.7 kg; $p = 0.006$). Multivariate regression analysis for maternal weight gain, neonatal length, neonatal weight and neonatal head circumference showed an independent correlation with maternal vitamin D level.
Conclusion: Treatment of low serum vitamin D during pregnancy improves fetal growth indices and maternal weight gain.

1. Introduction

Considerable changes in calcium homeostasis occur during pregnancy to meet the demands of the fetus. The fetal skeleton contains approximately 30 g of calcium at birth [1]. These changes are particularly evident in the third trimester, when almost 250 mg/day of calcium is transferred from mother to fetus [2]. Increased intestinal absorption is the main mechanism for supplying the increased demand for calcium [3]. Vitamin D and its active metabolite, $1,25(OH)_2D$, are the essential elements for intestinal calcium uptake. The concentration of $1,25(OH)_2D$ increases by 50–100% during the second trimester and 100% by the third trimester [4]. At present, the general recommendation for administration of vitamin D in pregnant women is 400 U/day. Some studies have shown, however, that this amount of vitamin D is insufficient during gestation, and higher doses are needed to prevent the complications associated with vitamin D deficiency during pregnancy [5].

Studies on pregnant women have demonstrated a high prevalence of vitamin D deficiency worldwide, particularly in developing countries and in women of Asian ethnicity [6–9]. Reports from Iran have shown vitamin D deficiency in 40–80% of pregnant women during different seasons of the year [10,11]. Descriptive studies have reported correlation between vitamin D deficiency and maternal complications (e.g. pre-eclampsia [12], decreased fertility [13] and increased bone resorption markers

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[14]) and fetal complications (e.g. higher risk of infection [15], neonatal hypocalcaemia [16] and craniotabes [17]), but findings regarding the association between vitamin D deficiency and decreased fetal growth indices are inconsistent [18–21]. Maternal ultraviolet B exposure is positively associated with bone mineral content and bone size of infants, suggesting that maternal vitamin D has a direct effect on periosteal bone formation of infants [22]. An association between low maternal vitamin D in late pregnancy and reduced intrauterine long bone growth has been reported [20]. However, the exact mechanism of action of maternal vitamin D deficiency on fetal growth indices is not clear. Neonatal vitamin D level correlates well with maternal vitamin D level [23]. Osteoblasts have 1,25(OH)D receptors, and low 25(OH)D and/or 1,25(OH)D in the fetus results in decreased osteoblast activity, reducing bone growth [24]. Fetal vitamin D deficiency can also stimulate fetal parathyroid hormone and parathyroid-hormone-related protein activity, leading to decreased cortical bone growth [25].

A few intervention studies have been undertaken to investigate the effect of vitamin D administration on fetal growth [26–28]. Some of these studies found no effect on fetal growth, while others reported an improvement in fetal growth indices. Given the high prevalence of vitamin D deficiency in Iran and the low number of intervention studies, this clinical trial was designed to investigate the effect of vitamin D treatment during the third trimester on fetal growth and maternal weight gain in women with vitamin D deficiency or insufficiency.

2. Materials and methods

This open-label randomized clinical trial was undertaken in Qazvin, Iran, from December 2011 to March 2012. It was approved by the Ethics Committee of Qazvin University of Clinical Science. Details of the study were explained to the participants, and written consent was obtained before participation. This study was registered retrospectively on www.irct.ir (Registration number: 201205119491N2).

The primary outcome was the effect of treatment of maternal vitamin D deficiency or insufficiency on newborn length at birth. Secondary outcomes were the effect of treatment on other fetal growth indices (head circumference and weight) and maternal weight gain during pregnancy. Vitamin D deficiency and insufficiency were defined as serum 25(OH)D level $<$20 ng/ml and 20–30 ng/ml, respectively [29]. In total, 160 pregnant women (24–26 weeks of gestation) who attended an obstetric clinic for antenatal care were investigated. Inclusion criteria were: gestational age of 24–26 weeks, singleton pregnancy and body mass index (BMI) of 19–26 kg/m^2. Exclusion criteria at study enrolment were: diabetes before pregnancy, chronic hypertension, history of repeated abortion, rheumatoid arthritis, parathyroid disorders, hepatic or renal diseases, and use of aspirin, anticonvulsive and immunosuppressive drugs. Subjects who developed gestational diabetes, severe pre-eclampsia, fetal anomaly, preterm delivery (before 37 weeks of gestation), oligohydramnios or polyhydramnios were excluded at the end of the study before statistical analyses were performed.

Initially, blood samples were collected from pregnant women to measure the serum vitamin D level; once the results were obtained, those with vitamin D deficiency or insufficiency [serum 25(OH)D $<$30 ng/ml] were included in the study. Participants were assigned at random into the intervention group or the control group. Allocation was concealed and randomization was performed using computer-generated random numbers. Assignment into groups was performed by an obstetrician responsible for antenatal care. This study was conducted in accordance with the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of Qazvin University of Medical Science.

Women in the control group received a multivitamin containing 400 IU vitamin D_3 plus 200 mg elemental calcium each day until delivery. Women in the intervention group received a weekly dose of 50,000 IU oral vitamin D_3 for 8 weeks (from 26 to 28 weeks of pregnancy) as well as the drug regimen (multivitamin and elemental calcium) given to the control group. The participants were visited once every 2 weeks during the second trimester and once a week in the third trimester; parameters such as weight, blood pressure, uterine fundal length, and use of vitamin D supplement and multivitamin were measured or checked. Following delivery, maternal and cord blood samples (5 ml) were taken after clamping and sent to the hospital laboratory to be centrifuged and kept frozen until use. Serum vitamin D was determined using a commercial enzyme-linked immunosorbent assay kit (Euroimmun, Lubeck, Germany). The intra-assay and interassay coefficients of variation for 25(OH)D were 3.3% and 6.7%, respectively. On admission for labor, maternal weight, neonatal weight, neonatal length and neonatal head circumference were measured. Neonatal weight and length were measured using a calibrated instrument (Seca Medical Measuring Systems). Head circumference (largest occipitofrontal circumference) was measured to the nearest 1 mm using an unstretchable tape measure. Anthropometric measurements were taken by a nurse who was blinded to the patient's group.

Data were presented as frequency tables, diagrams and numerical indices. Chi-squared test, Fisher's exact test, ANCOVA test and *t*-test were used for data analysis. A multivariate regression analysis was performed to examine the independent effect of maternal vitamin D level on fetal growth indices.

3. Results

Vitamin D deficiency or insufficiency was found in 130 out of 160 pregnant women (81%). During the study, one case of gestational diabetes and one case of premature rupture of membranes occurred in the intervention group. Ten women in the control group and nine women in the intervention group were lost to follow-up as they gave birth in places that were difficult to access (Fig. 1).

Complete results were obtained for 54 women in the control group and 55 women in the intervention group. Mean gravidity, gestational age at delivery, BMI, sun exposure time and serum vitamin D level did not differ significantly between the two groups (Table 1). Gestational age at delivery was similar in both groups.

At delivery, maternal serum vitamin D level was significantly higher in the intervention group compared with the control group (Table 2). Mean neonatal length was 0.8 cm more in the intervention group compared with the control group ($p = 0.01$) (Table 2), and mean neonatal head circumference was also higher in the intervention group ($p = 0.001$) (Table 2). Finally, on average, neonates in the intervention group weighed 170.2 g more than neonates in the control group ($p = 0.01$) (Table 2). Women in the intervention group gained more weight during pregnancy compared with women in the control group ($p = 0.006$) (Table 2). A multivariate regression analysis was performed for each growth index and maternal weight gain to investigate independent correlation of fetal growth indices and maternal weight gain with maternal vitamin D level and other maternal factors (including age, BMI, gravidity, gestational age at delivery). Maternal vitamin D level and gestational age at delivery were independently correlated with neonatal length, weight and head circumference (Table 3). Maternal weight gain was independently correlated with serum vitamin D level, and inversely correlated with maternal BMI at enrolment.

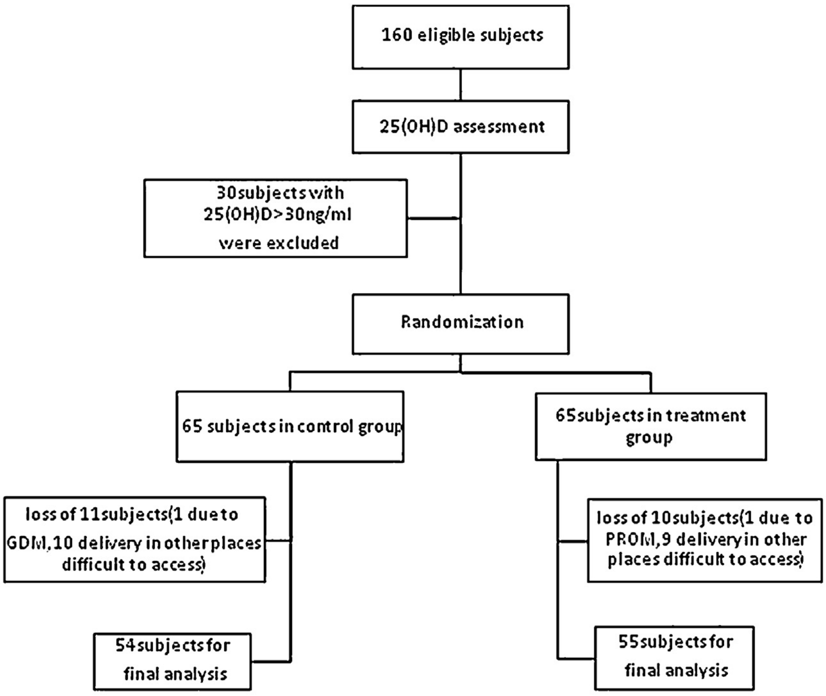

Fig. 1. CONSORT flowchart of participants.

Table 1
Baseline demographic and reproductive variables by group*.

	Control group		Intervention group	
	Total	Participants who completed study	Total	Participants who completed study
Number	65	54	65	55
Age (years)	27.4 ± 4.6	27.6 ± 4.6	26.9 ± 4.7	27.0 ± 4.6
Sunlight exposure <30 min/day (%)	50.7%	48.1%	49.2%	47.2%
Body mass index (kg/m^2)	24.5 ± 2.4	24.4 ± 2.5	24.5 ± 2.5	24.2 ± 2.5
Vitamin D level (ng/ml)	17.6 ± 4.8	17.5 ± 4.8	15.9 ± 5.6	15.8 ± 5.6
Vitamin D deficiency (<20 ng/ml) (%)	66.1%	62.9%	67.6%	67.3%
Vitamin D insufficiency (20–30 ng/ml) (%)	33.9%	37.1%	32.4%	32.7%

Values are given as mean ± standard deviation unless otherwise indicated.
* There were no significant differences in baseline characteristics between the two groups.

Table 2
Comparison of maternal and neonatal serum vitamin D levels and anthropometric indices of the two groups at the end of the study.

Variable	Control group (95% CI)	Intervention group (95% CI)	Mean difference (95% CI)	*p*-value
Maternal vitamin D (ng/ml)[a]	15.9 ± 6.6 (14.1–17.8)	47.8 ± 11.1 (44.8–50.8)	31.8 (28.3–35.3)	
Maternal vitamin D >30 ng/ml (%)	3%	100%		0.0001[b]
Neonatal vitamin D (ng/ml)[a]	10.9 ± 4.4 (9.4–11.9)	27.7 ± 5.2 (26.3–29.1)	17.0 (15.2–18.9)	0.001[c]
Neonatal vitamin D >30 ng/ml (%)	0%	32.7%		0.0001[d]
Neonatal length (cm)[a]	48.2 ± 1.7 (47.7–48.6)	49.0 ± 1.6 (48.6–49.4)	0.8 (0.18–1.4)	0.01[c]
Neonatal head circumference (cm)[a]	35.3 ± 1.0 (35.0–35.6)	35.9 ± 0.7 (35.7–36.1)	0.6 (0.2–0.9)	0.001[c]
Neonatal weight (g)[a]	3258.8 ± 328.2 (3169.2–3348.4)	3429.0 ± 351.9 (3333.9–3524.2)	170.2 (40.9–299.4)	0.01[c]
Maternal weight gain during pregnancy (kg)[a]	11.7 ± 2.7 (11.0–12.5)	13.3 ± 2.4 (12.6–13.9)	1.5 (0.5–2.4)	0.006[e]

CI, confidence interval.
[a] Values are given as mean ± standard deviation.
[b] Significant difference on Chi-squared test.
[c] Significant difference on *t*-test.
[d] Significant difference on Fisher's exact test.
[e] Significant difference on ANCOVA test; maternal weight gain adjusted for body mass index before intervention.

Table 3
Multivariate regression analysis of predictors of fetal growth indices.

	Maternal serum vitamin D		Gestational age		Body mass index[a]	
	p	Beta	*p*	Beta	*p*	Beta
Neonatal length	0.008	0.24	0.001	0.32	0.646	−0.04
Neonatal head circumference	0.001	0.31	0.005	0.26	0.574	0.05
Neonatal weight	0.002	0.28	0.003	0.28	0.267	0.1
Maternal weight gain	0.01	0.23	0.351	−0.08	0.007	−0.26

Maternal age, gravidity, body mass index, gestational age and serum vitamin D level were entered into the model. Maternal age and gravidity were not significantly correlated with fetal growth indices or maternal weight gain during pregnancy.
[a] Body mass index before intervention.

No cases of neonatal death or congenital malformations occurred in either group. One neonate in the control group was small for gestational age, and all neonates in the intervention group were of normal weight.

4. Comments

This study found that treatment of pregnant women with vitamin D deficiency or insufficiency resulted in improved fetal growth indices (length, head circumference and weight) and greater maternal weight gain during pregnancy. In addition, maternal vitamin D level was significantly independently correlated with neonatal length, weight and head circumference.

Most studies undertaken on the association between maternal vitamin D level and fetal growth indices have been descriptive cross-sectional studies, and the results have been inconsistent. Some of these studies found no correlation between vitamin D level and bone density or anthropometric characteristics [21], whereas other studies found that fetal growth indices were lower in cases where the mother was vitamin D deficient [17]. Few clinical trials have been undertaken regarding the effect of vitamin D administration in pregnant women on fetal growth. Kalra et al. reported improved anthropometric characteristics (length, weight, head circumference and fontanelle size) in neonates following administration of a single dose (1500 μg) of vitamin D during the second trimester, or two doses (3000 μg) of vitamin D during the second and third trimesters [26]. In another study by Marya et al. on pregnant women who received two doses of vitamin D (600,000 U) during the seventh and eighth months of gestation, neonatal weight and serum calcium and phosphorus levels were higher, but alkaline phosphatase levels were lower [27]. On the contrary, Mallet et al. investigated the effect of vitamin D administration on biochemical parameters and weight in three groups of pregnant women [Group I: vitamin D (5 mg) at 7 months of gestation; Group II: vitamin D (1000 U/day); Group III: no vitamin D administration], and found no significant difference in neonatal weight between the groups [28].

The discrepancies in clinical trial results could be associated with differences in the baseline vitamin D levels of the pregnant women, and different doses of vitamin D administered. In the present study, the main inclusion criterion for pregnant women was maternal serum 25(OH)D level <30 ng/ml, whereas in other studies, this measure was not taken into account. The inclusion of pregnant women with a normal level of vitamin D in clinical trials could dilute the results of the study and eventually lead to an insignificant difference in the findings.

The present study had strengths and weaknesses. Strengths included the intervention study design, recruitment in a single season, and the inclusion criterion of vitamin D deficiency or insufficiency. A limitation of the present study was the lack of use of a placebo, although the investigator who evaluated the fetal growth indices was blind to the mothers' group allocation.

In conclusion, this study showed that treatment of pregnant women with vitamin D deficiency or insufficiency improves fetal growth indices (length, weight and head circumference) and increases maternal weight gain during pregnancy.

Conflict of interest

None declared.

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